Attorney Docket No. P63142US1 Application No. 09/806,509

Amendments to the specification:

Rewrite page 29, Ist paragraph, as:

Figure 2 depicts Figures 2A and 2B depict the influence of freezing and thawing of CSF samples on their cystatin C level.

Rewrite page 39, 3rd paragraph, as:

Figure 2 depicts Figures 2A and 2B depict the influence of repeated thawing and freezing CSF samples on their cystatin C content. Figure 2a 2A shows the inter-individual variability of the sensitivity to the number of thaw-freeze cycles. In some samples, levels of the cystatin C monomer decreased 50% after two cycles, whereas in others it remained stable. After four or five freezing and thawing cycles, however, all samples consistently had significantly lower levels of the monomeric form of cystatin C. Figure 2b 2B shows cystatin C levels after 1 and 4 freezing-thawing cycles, respectively.

Rewrite the paragraph bridging pages 12 and 13 as:

Determining the presence or absense absence of polymorphism in a cystatin C gene in a sample from said subject may comprise determining a partial nucleotide sequence of the DNA from said subject, said partial nucleotide sequence indicating the presence or absence of said polymorphism. It may further be preferred to perform a polymerase chain reaction with the DNA from said subject and subsequent restriction analysis to determine the presence or absence of a polymorphism. Such techniques are known to those of ordinary skill in the art (see Lewin, B., Genes V, Oxford University Press, 1994). In a further preferred embodiment, primers depicted in SEQ ID NO: SEQ ID NO: 3 and SEQ ID NO: SEQ ID NO: 4 are used for amplifying parts of the promoter region as well as the

Attorney Docket No. P63142US1 Application No. 09/806,509

coding sequence of exon 1 of the human cystatin C gene in order to subsequently analyze the Sst II polymorphic sites herein.

Rewrite the paragraph bridging pages 15 and 16 as:

Determining the presence or absense of a mutation or polymorphism in a cystatin C gene in a sample from said subject may comprise determining a partial nucleotide sequence of the DNA from said subject, said partial nucleotide sequence indicating the presence or absence of said mutation or polymorphism. It may further be preferred to perform a polymerase chain reaction with the DNA from said subject and subsequent restriction analysis to determine the presence or absence of said mutation or polymorphism. Such techniques are known to those of ordinary skill in the art (see Lewin, B., Genes V, Oxford University Press, 1994). In a further preferred embodiment, primers depicted in SEQ ID NO: SEQ ID NO: 3 and SEQ ID NO: SEQ ID NO: 4 are used for amplifying parts of the promoter region as well as the coding sequence of exon 1 of the human cystatin C gene in order to subsequently analyze the Sst II polymorphic sites herein.

Rewrite page 32, 1st complete paragraph, as:

A Keyhole himpet hemosiderin-conjugated peptide of SEQ ID NO: 1 (EGDPEAQRRVSKNSK) was used to immunize rabbits with to generate the antiserum R9672. The antiserum was affinity-purified after ammonium sulfate precipitation by binding to the above peptide conjugated to NHS-activated sepharose (HiTrap, Pharmacia). In CSF, this antiserum specifically detected the monomer form of cystatin C that migrated on 2D gels at a pH of 8.75, with a molecular mass of 13.3 kDa, the identical protein was detected by a commercial anti-human cystatin C antibody (Amava, Switzerland). The signal generated by either antibody was preadsorbable by preincubation of the antiserum with puried purified cystatin C, and densitometric measurements of signal intensities generated with human CSF samples correlated significantly (r = 0.952, P < 0.003).

Attorney Docket No. P63142US1 Application No. 09/806,509

Together, the characterization of R9672 revealed that it specifically reacted in CSF with cystatin C, despite the fact that it was raised against, and detects in brain protein extracts, the 21 kDa presentin 1 C-terminal fragment.

Rewrite the paragraph bridging pages 36 and 37 as:

The R9672 Western blotting assay was used to purify the 13.3 kDa protein from human CSF by combined ion exchange chromatography and SDS-polyacrylamide electrophoresis to determine its N-terminus by automated Edman degradation sequencing. Sequence analysis unequivocally revealed SSPGXPPRLVGGPMXA (SEQ ID NO: SEQ ID NO: 2), corresponding with 100 % identity to the N-terminus of cystatin C without the signal peptide. There were no additional sequences detectable in this preparation.

Rewrite page 37, 2nd full paragraph, as:

By use of polymerase chain reaction (PCR), a 318 bp segment that comprises parts of the putative promoter region of the cystatin C gene as well as the coding sequence of exon 1 (see Fig. 3), has been amplified (see Fig. 3 regarding mutations in the promoter region; an Ala/Thr exchange in the coding region of the human cystatin C gene has been detected as a Sst II polymorphism by Balbin et al., Hum. Genet. 92: 206 - 207, 1993). The primers used had sequences 5'-TGGGAGGGACGAGGCGTTCC-3' (SEQ ID NO: SEQ ID NO: 3) and 5'-TCCATGGGGCCTCCCACCAG-3' (SEQ ID NO: SEQ ID NO: 4). Amplification was carried out in a thermocycler under standard conditions without additives. PCR products were digested with Sst II or its isoschizomer Sac II for 1 hr at 37 °C and separated by 4 % agarose gel electrophoresis. CST-3 B alleles derived restriction fragments are apparent in this system with 127 and 191 bp in length. A2M and ApoE were genotyped according to standard protocols.